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The goals of this project are to characterize the anatomical pathways and neurotransmitters that participate in the brain neuroendocrine stress response. Studies have focused on the amygdala, a part of the limbic system. Specifically, studies are designed to 1) determine if and which ACTH releasing cells of the hypothalamus are innervated by the amygdala; 2) determine the neurotransmitter used by cells within the amygdala to activate the release of ACTH; and 3) determine the role of the amygdala in release of ACTH and related "stress" hormones to different psychological and physical stressors. During the first year, light microscopic studies on the amygdala-hypothalamic pathways have been completed. The amygdala topographically innervates subregions of the hypothalamic paraventricular nucleus. Amygdaloid terminals appear to contact CRF, vasopressin and oxytocin immunoreactive cells. Studies are in progress to determine if destruction of the amygdala will affect neuroendocrine responses to immobilization or cold stress. Studies on the identification of amygdaloid (cont.)

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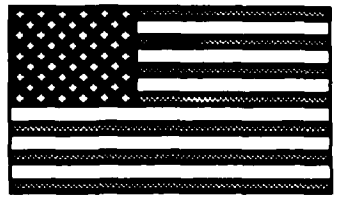

Date: December 1, 1988 **R & T Code: 4414809-01**

ANNUAL REPORT ON CONTRACT ONR N00014-88-K-0010

PRINCIPAL INVESTIGATOR: Thackery S. Gray

CO-INVESTIGATOR: Louis D. Van De Kar

CONTRACT TITLE: ***Amygdalo-hypothalamic Pathways Mediate Stress-induced ACTH Release.***

Contract Period: Dec. 1, 1987 to Nov. 30, 1988

RESEARCH OBJECTIVES: To characterize the anatomical pathways and neurotransmitters that participate in the brain neuroendocrine stress response. We have focused our study on the amygdala which is composed of a heterogeneous group of nuclei and is part of the limbic system. Our studies are designed to determine which ACTH releasing cells of the hypothalamus are innervated by the amygdala, to determine the neurotransmitter used by cells within the amygdala to activate the release of ACTH, and to determine the role of the amygdala in release of ACTH and related "stress" hormones to different psychological and physical stressors.

PROGRESS REPORT: A series of inter-related studies on the anatomy, physiology and neurotransmitters of the amygdalo-hypothalamic pathway have been initiated. **First,** we have completed one study that characterizes the anatomical organization of the pathway from the amygdala to the paraventricular nucleus of the hypothalamus. The results of this study demonstrate that the amygdala directly innervates the posterior and anterior parvocellular regions of the paraventricular hypothalamic nucleus. It is the central amygdaloid nucleus that projects to the posterior parvocellular subregions of the paraventricular nucleus. The posterior parvocellular regions of the paraventricular nucleus

contain the corticotropin releasing cells of the hypothalamus (i.e., corticotropin releasing factor, vasopressin and oxytocin). The data was presented in preliminary form at the Molecular Biology of Stress Meetings in Keystone, Colorado, April 10-17 (see abstract). A manuscript was submitted to Neuroendocrinology and has been accepted pending revision. **Second,** during the summer we have developed a double labeling technique that will allow us to determine which ACTH releasing cells are being innervated by the amygdala. Our initial data using this technique shows that the central amygdaloid axon terminals directly contact parvocellular corticotropin releasing factor, vasopressin and oxytocin cells. Magnocellular CRF, vasopressin and oxytocin cells were not strongly innervated by the amygdala. Thus, this amygdalo-hypothalamic pathway is selectively linked to cells that have established ACTH releasing properties. This data was presented at the Society For Neuroscience Meetings at Toronto, Canada in November. **Third,** in February and March of this year the principal investigator visited Dr. Marvin Brown's lab at the University of California at San Diego. There we conducted studies to determine if thyrotropin releasing factor (TRF), a central nervous system stress-related peptide "neurotransmitter", could effect the release of ACTH. We suspected that it may have ACTH releasing properties, because it has potent stress-related cardiovascular activating effects when injected into the amygdala or cerebral ventricles. The data that we have collected so far demonstrate that TRF injected into lateral ventricle of the brain does indeed increase plasma levels of ACTH. This is the first evidence demonstrating ACTH releasing properties of thyrotropin releasing factor, although its effect upon pituitary release of other hormones is well established. The TRF-induced ACTH response was not blocked by intracerebroventricular injection of CRF antagonist alpha-helical CRF 9-41. However, the ACTH response was totally blocked by IV injections CRF antisera (passive immunization). Thus, TRF probably effects the release of ACTH from the pituitary by activating hypothalamic CRF neurons that project to the median eminence (portal release). The data was presented at the Society For Neuroscience Meetings at Toronto, Canada in November. Future studies will be conducted to determine if the amygdala is one of the sites of action for TRF-induced ACTH release. **Fourth**, experiments on the effects of amygdaloid lesions on neuroendocrine responses to cold stress, hemorrhagic stress and psychological (i.e., immobilization or tone signaling shock) stress are underway in Dr. Van de Kar's laboratory. In the initial control experiments, ibotenic acid induced-lesions were placed dorsal, ventral and lateral to the amygdala. These lesions failed to have any effect upon cold water-

and forced swimming-induced increases in plasma corticosterone and renin concentrations. Additional experiments are in progress to determine if lesions of the central amygdala will block the corticosterone and renin response to this stress. In another initial experiment, the catecholamine neurotoxin, 6-hydroxydopamine, was injected into the central amygdala of rats. The rats were pretreated with desipramine to protect noradrenergic terminals, but allowing the dopaminergic terminals to be selectively lesioned. Lesions of the dopaminergic terminals within the amygdala blocked the effect of "psychological" stress-induced increases in plasma renin and corticosterone. The psychological stress was exposure to a cage in which the animals had previously been exposed to mild foot shock. Placement in the cage caused significantly elevated levels of the plasma renin and corticosterone in sham-lesioned controls. Thus, these preliminary results implicate an important role for the dopamine in amygdaloid mediated ACTH and renin responses to stress. These experiments are being conducted by Peter Rittenhouse who is receiving his Ph.D. training in Dr. Van de Kar's laboratory.

EXPENDITURES:

Supplies: expenditures normal

Equipment: ONR Funds were used to purchase an Olympus Vanox microscope.

Travel: see below.

1. The PI (Thackery S. Gray) was funded to engage in collaborative research in Dr. Marvin R. Brown's laboratory at the University of California at San Diego from February 21 to March 19, 1988.

2. Partial funding of the PI's trip to Molecular Biology of Stress Meetings in Keystone Colorado April 10-17, 1988.

3. Full funding of the PI's and CO-PI's trip (Louis Van De Kar) to the Society For Neuroscience Meeting in Toronto Canada November 12-18, 1988.

Other: expenditures normal.

Personnel: Rashel Piechowski: Research Assistant I (100% support from ONR)

Joseph Costello: Research Assistant I (approx. 50% support from ONR)

Training Activities: Peter Rittenhouse is currently receiving his Ph.D training in Dr Van De Kar's lab. The present ONR contract only supports the portion of his research that is directly related to the goals of the project. The support is in the form of paying for supplies and animal care.

Women or minorities - 0; Non-citizens - 0

PUBLICATIONS AND REPORTS:

Abstracts

Gray, T.S. and M. Carney Demonstration of direct projections from the amygdala to the paraventricular hypothalamic nucleus: Possible role in stress induced ACTH release. **J. Cellular Biochemistry**, 12D (1988) Q302.

Brown, M.R., C. Rivier and T.S. Gray Thyrotropin-releasing factor (TRF) effects on pituitary ACTH release and autonomic function, Soc. **Neurosci. Abstr.** 14 (1988) 1287.

Magnuson, D.J. and T.S. Gray Amygdala directly innervates parvocellular paraventricular hypothalamic CRF, vasopressin and oxytocin. Soc. **Neurosci. Abstr.** 14 (1988) 1288.

Manuscripts

Gray, T.S., M.E. Carney and D.J. Magnuson Direct projections from the central amygdaloid nucleus to the hypothalamic paraventricular nucleus: Possible role in stress-induced ACTH release. **Neuroendocrinology**, accepted pending revisions.

2ND YEAR WORK PLAN: The objectives of Year 2 are **1)**. to complete the above studies and submit them as publications; **2).** to begin electron microscopic studies on the amygdala-hypothalamic pathway. Electron microscopic techniques are necessary to prove that the terminals we have observed on hypothalamic "CRF" cells

form synaptic contacts; **3).** to determine which "neurotransmitter" candidates are contained with the amygdaloid cells that directly innervate hypothalamic CRF cells. This will be accomplished via combined anatomical retrograde tracing and immunohistochemical experiments, and through continued collaborative efforts with Dr. Marvin R. Brown; **4).** to continue and complete studies on the effects of neurotoxic lesions of the amygdala on the expression of ACTH responses to stress. Other stress models such as immobilization and hemorrhagic stressors will be examined.

NOTE TO SCIENTIFIC OFFICER:

1). The expenditures for year 1 correspond to original expectations except for our purchase of an Olympus AH2 Vanox microscope. The purchase of this microscope was necessary for processing of the two color immunocytochemical histological and fluorescent anatomical tracing material collected for completion of the ONR contracted experiments. The Olympus AH2 (which we compared and found superior to our present and other microscopes) cost $35,865.. The ONR budgeted $19,000 for the microscope. We still were able to purchase the microscope by using the $19,000, plus $9,000 from the ONR personnel budget and $7,865 from the PI's NIH grant. Funds became available in the personnel part of the budget for two reasons: 1) there was a delay in funding of the contract [start date Dec. 1, 1987 and funding actually started the last week of January, 1988]; 2) there was delay in hiring a part time research assistant because we had difficulty finding a qualified person to fill the position. I would like to note that the Olympus AH2 microscope has been a tremendous boost to the anatomical studies of the ONR contract. The histological material would have been difficult to process and publish without this instrument.

2). I will be on sabbatical from January 15 to June 15, 1988. During my absence Dr. Van de Kar will still be on site to conduct studies and provide guidance for the personnel of the ONR project. Dr. Anthony Castro (Dept. Anat., adjacent laboratory) has consented to also check in on my laboratory regularly.

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